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Antimicrobial Activity Of Clove Against Escherichia Coli And Candida Albicans İsolated From Urinary İnfections İn Women

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Abstract

Urinary tract infections (UTIs) are one of the most frequent antimicrobial infections in women and men of all ages over the world. Syzygium aromaticum has been used in traditional medicine to treat microbial infections of the skin, mouth, urinary and vaginal tract. To evaluate Syzygium aromaticum for their antimicrobial activity against *Escherichia coli* and *Candida albicans* isolated from urinary tract infections. For this purpose,116 urine samples from women who were the subject of a complaint of urinary tract infection with ages ranging between (1-86) years. The recognition of microbial isolates was based on their physiological and morphological characteristics. Furthermore, The antimicrobial activity of Syzygium aromaticum against *Escherichia coli* and *Candida albicans* was investigated using the agar dilution method, were utilized in order to assess the minimum inhibitory concentration (MIC), the minimum bactericidal concentration (MBC) and the minimum fungicidal concentration (MFC). In addition, disk diffusion and agar well diffusion method for determination of the zone of inhibition. Data were analyzed using a t-test with one-way analysis of variance (ANOVA), and the results were shown as the mean ± standard deviation (SD) by SPSS software and value of $p<0.05$ was considered statistically significant The results of this study indicated that *C. albicans* is less resistant to the cloves than the *E. coli*. Also, we observed that powder S. aromaticum have remarkable potential as an antimicrobial agent, deserving further investigation for clinical applications..

Keywords: S. aromaticum, *C. albicans*, *Escherichia coli* , urinary tract , infections

Introduction

Clove (Syzigium aromaticum L., family Myrtaceae) is used as a food preservative and a medicinal plant mainly Because of its antioxidant and antimicrobial properties. Many studies now show that spice plants have antibacterial, antifungal, antiviral, and anticarcinogenic effects. So clove has gotten a lot of attention due to activities standing out among the other spices (Cortés-Rojas, de Souza, dan Oliveira 2014; Shan et al. 2005). Commercial cloves are the dried and unexpanded flower buds used as a condiment or spice in various foods and pharmaceuticals (Cai dan Wu 1996; El-Maati et al. 2016; Ramadan, Asker, dan Tadros 2013). Clove contains many bioactive compounds such as triterpenoids and sesquiterpenes(Ramadan et al. 2013). Eugenol (4-allyl-2- methoxyphenol), the major bioactive compound of clove oil, displayed strong

insecticidal (El-Maati et al. 2016; Park et al. 2000). The essential oil extracted from the buds of S. aromaticum is widely utilized in medicine, particularly in dental treatment. The essential oil is effective against oral bacteria associated with dental caries and periodontal disease(Cai dan Wu 1996; El-Maati et al. 2016; Ramadan et al. 2013) ,and in medical dental practices as a general antiseptic (Ahmad et al. 2005; Cai dan Wu 1996; Rana, Rana, dan Rajak 2011a) . Also, as a carminative, anaesthetic, and as an antimicrobial for skin infections(Cai dan Wu 1996; Ghelardini et al. 2001; Karmakar et al. 2012; Khan dan Ahmad 2012). In addition, used for dyspepsia and gastric irritations(Fu et al. 2007; Jain dan Pundir 2010) .As well as, a perfume and food flavouring(Zheng, Kenney, dan Lam 1992), as a medicine to treat asthma and various allergic disorders(Kim et al. 1998; Rana et al. 2011a). Herbal extracts have a lengthy history of application in the treatment of several human ailments. According to a World Health Organization (WHO) assessment, more than 80% of the world's population rely on traditional medicine for their primary healthcare requirements (Javidnia et al. 2009). It had been at present proposed that laboratory purified components of herbal extracts may cause some side effects and if they accompanied other components found in the extract, these side effects would be eliminated(Mahboubi et al. 2009; Mansourian et al. 2014). Medical plants, spices, herbs and oilseeds are rich sources of natural antioxidants like curcuminoids, flavonoids, tannins, terpenoids and lignans. There is interest in characterizing and extracting natural bioactive compounds from these sources for using them in various food and pharmaceutical applications(Dhaouadi et al. 2015; El-Maati et al. 2016; Remila et al. 2015).Urinary tract infections (UTIs) are among the most common infections affecting humans today (Yadav et al. 2014). Bacteria is the most common cause of these infections but other microbes, including fungus, parasites, and protozoa, also cause urinary tract infections(Stone dan May 1983). The presence of bacteria in the urinary system results in serious infections that can cause kidney failure, and in some cases death(Matchar et al. 2002). Escherichia coli is considered, is the most common infecting agent in the urinary tract, which primarily affects neonates, preschool girls, sexually active women, and elderly women(Liu et al. 2008a).Escherichia coli is the causative factor as the etiologic agent in at least 75% of women who present symptoms of cystitis. E. coli also responsible for 90 to 100% of bacterial infections in the kidneys or acute pyelonephritis.C. albicans is an opportunistic, polymorphic yeast that colonizes 30 to 70% of human skin, genital, and intestinal mucosae. Generally, C. albicans doesn’t cause infection, but when host immune functions are decreased or the competitive commensal flora is perturbed, infections can cause(Ayaal dan Chehri 2019; Lewis et al. 2012). A study conducted in the United States has showed that C. albicans infections are the fourth most prevalent hospital-acquired systemic infections. They are also considered to responsible for a high mortality rate(Pfaller dan Diekema 2010). Other bacterial species, such as Pseudomonas aeruginosa, Klebsiella spp., Enterococcus spp., and Proteus mirabilis, can cause UTIs (Lewis et al. 2012) Women are more likely than men to develop urinary tract infections(Foxman dan Brown 2003; Smelov, Naber, dan Bjerklund Johansen 2016). In recent years, a rapid increase in microbes that are resistant to conventionally-used antibiotics has

been observed(Kim et al. 2009).The use of antibiotics to treat infections can also become a major environmental problem since the human body does not process these substances completely, and therefore they persist in wastewater (Liu et al. 2008b).The development of microbial resistance to antibiotics is a major challenge in the medical field. Therefore, the search for drugs with new modes of action is of major interest in the pharmaceutical and research communities .Medicinal plants and nanomaterials are two potential sources of new antibacterial agents(Senarathna et al. 2017). Plant-derived compounds have been researched as disease control agents because they have low mammalian toxicity, minimal environmental consequences, and widespread public acceptance (Benincasa et al. 1990; Rana, Rana, dan Rajak 2011b).A large number of herbs possess antimicrobial activity(Mothana dan Lindequist 2005; Voravuthikunchai et al. 2004), and some active components of them have become a potential source of new anti-infective agents(Agunu et al. 2005; Buwa dan Van Staden 2006; Fu et al. 2007). The aim of this study was an assessment of in vitro antibacterial and antifungal activity S. aromaticum extracts in comparison with Amikacin on E. coli and Fluconazole on C. albicans samples isolated from urinary infections in women.

Materials and Methods

Plant material

The buds of S. aromaticum (clove) were obtained from a local market in Kermanshah, Iran. It was grinded by the electric grinder. It was sifted by a small sieve, and then assembled into a sterile glass tube and stored at laboratory temperature.

Organisms

In this study, 116 urine samples from women who were the subject of a complaint of urinary tract infection who attending Imam Reza Hospital in Kermanshah were taken with ages ranging between (1-86) years. All urine samples were plated on Mac Conkey and Blood agar plates and incubated at 37 for 24 - 48 hours. Identification of pure isolate was done by observing morphological, cultural and biochemical characters. After incubation, the plates were examined for bacterial growth and quantified. For positive cultures, bacterial identification was done using morphological characteristics, Gram staining and various biochemical tests(Oscarson 2014). Escherichia coli was seen in 32 patient samples.While recognized of C. albicans isolates was based on their physiological and morphological characteristics,in addition to making The germ tube test (GTT) was carried out by picking a pure colony from SDA agar with a sterile swab . The colony was suspended in 0.3-0.5 ml human serum at room temperature. Serum cultures was incubated at 37 °C for 2.5-3 hours. A drop of the serum culture was placed on a clean slide and examined under the microscope using low and high powers. Formation of germ tubes was observed in positive isolates shown in (Fig. 1). A single colony from SDA plates was streaked out on CHROMagar Candida and incubated for 24-48

hours at 30-35 °C. C. albicans turned green in this medium and were isolated from other species of Candida with different colours. C. albicans were seen in 28 patient samples.

In vitro antimicrobial assay

Agar Dilution Method

The susceptibility test was studied with a conventional serial agar dilution method with some modification. EMB agar medium used as the culture medium instead of Muller-Hinton agar, (MHA) for E. coli. For C. albicans, Sabouraud Dextrose Agar (SDA) The EMB and Sabouraud Dextrose agar medium were further supplemented with serial concentrations of S. aromaticum powder (200, 150, 100, 50, 25, 12.5 mg). An inoculum of approximately 1.5 × 108 colony-forming units (Cfu) per millilitre of E.coli and 106 of C. albicans, applied on the surface of the S. aromaticum powder supplemented (EMB) and (SDA) agar plates. Inoculated plates were incubated at 35°C for 24- 48 h. The MIC was determined as the lowest concentration of S. aromaticum powder inhibiting the visible growth of each organism on the agar plate(Sherwani et al. 2011).

Determination of zone of inhibition

Disk Diffusion Method

Filter paper discs Whatman No.1 of 5 mm were impregnated for 10 minutes in 100µl of each concentration separately (500, 250, and 125 ppm of S. aromaticum), Fluconazole (control antifungal), Amikacin 30mcg (control antibacterial. The prepared discs were dried by heating at 40 °C for one hour. Then, one ml of suspensions from C. albicans and from E. coli was added to the Mueller Hinton agar. The plates were gently rotated to mix the content and allowed to solidify at room temperature. On the surface of the plates, the paper discs of the drugs were pressed firmly for complete contact with the agar. The plates were incubated at 37ºC for 24 hrs. After the end of incubation period, the sensitivity of fungi or bacteria to each tested drug was determined by measuring the diameter of the growth inhibition zone in mm around discs(Atef dan Oraby 2015).

Well diffusion method

The Agar-well method is the method, which was used to evaluate the antibacterial and antifungal activity of Clove. Use to prepare stock solutions by dissolving from S. aromaticum in distilled water. The swabbing is done to make the lawn of culture by using two hours old log phase culture turbidity of which is matching with 0.5 Mac Farland and then wells were made in Muller Hinton agar. 100 µL of stock solutions were added into the wells(Perez, Pauli, dan Bazerque 1990). The plates were incubated at 37 °C for 24 -48 hours and results are noted by measuring the diameter of zone of inhibition in mm. The ruler was used to measure the inhibition zone. Fluconazole was employed as a positive control for C. albicans and Amikacin, (control antibacterial), DMSO is taken as negative control(Vaghasiya et al. 2009).

Statistical analysis

Statistical analysis of the results was performed using one-way analysis of variance (ANOVA), with at least three independent results. With T-test and the results were shown as the mean ± standard deviation (SD) by SPSS software (version 25) and value of $p<0.05$ was considered statistically significant. Compared with the positive control**.**

Results

The Antimicrobial activity of the dried flower buds of Clove (S. aromaticum) showed variable activity against E. coli and C. albicans organisms. The minimum inhibitory concentration of the (S. aromaticum) was determined against the E. coli, and C. albicans using the agar diffusion method with a concentration of (200 mg, 150mg, 100mg, 50mg, 25mg and 12.5mg), and the results were summarized in (Table 1). The S. aromaticum showed that 50 mg was the minimum concentration to inhibit the growth (MIC) of E. coli, and showed that 25 mg was the minimum fungal concentration of the C. albicans. The results were summarized in (Table 4.5). The MBC of the S.aromaticum was 100 mg against E. coli while The MFC 50 mg for C. albicans. To determination of zone of inhibition used disk diffusion method, as shown in (Fig 2– 3). S. aromaticum the higher antibacterial activity with inhibition zone of 15.67 ± 0.577 and antifungal activity with inhibition zone of 17.67 ± 3.55 as shown in (Table 2).

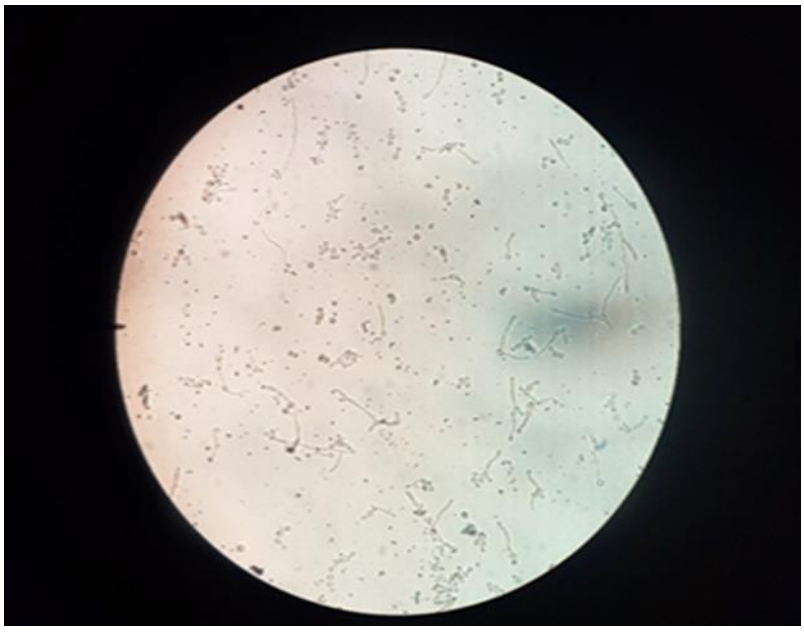


Figure1: Micrograph showing formation of germ tubes by C. albicans grown in Serum for 3 hours at 37 °C

Table 1: The in vitro antimicrobial effects of S. aromaticum against E. coli and C. albicans.

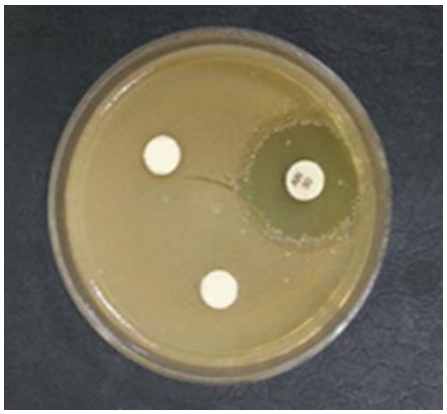
Nano particles	E. coli		C. albicans	
	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MFC (mg/ml)
S. aromaticum	50 ± 0	100 ± 0	25 ± 0	50 ± 0

Notice: The results (mean ± standard deviation; E. coli n=32, C. albicans n=28) in columns with various letters are significantly different at P< 0.05. MIC=Minimum inhibitory concentration (mg/ml); MBC=Minimum bactericidal concentration (mg/ml). MFC=Minimum fungal concentration (mg/ml).

Table 2 : Sensitivity Testing of Organisms with Standard Deviation in Zones of Inhibition (Disk Diffusion Test).

Materials	Organisms/Mean zone diameter (mm) ± SD	
	E. coli	C. albicans
S. aromaticum	15.67 ± 0.577	17.67 ± 3.55
Amikacin	21±0	__
Fluconazole	__	15.33 ± 2.94

Notice: Control-, Amikacin (Bacteria) and Fluconazole (Fungi), mean zone inhibition (mm) ± standard deviation of three replicates, P < 0.05



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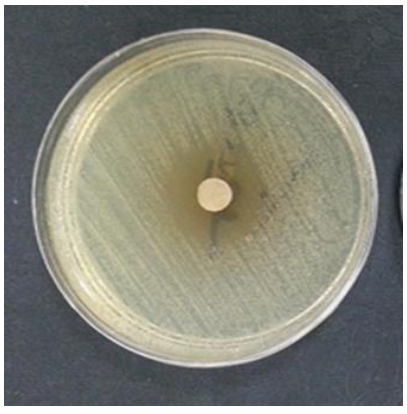

Figure 2.Antibacterial activity of S. aromaticum combined effect with antibiotics (A) Amikacin, (B) S. aromaticum against E. Coli

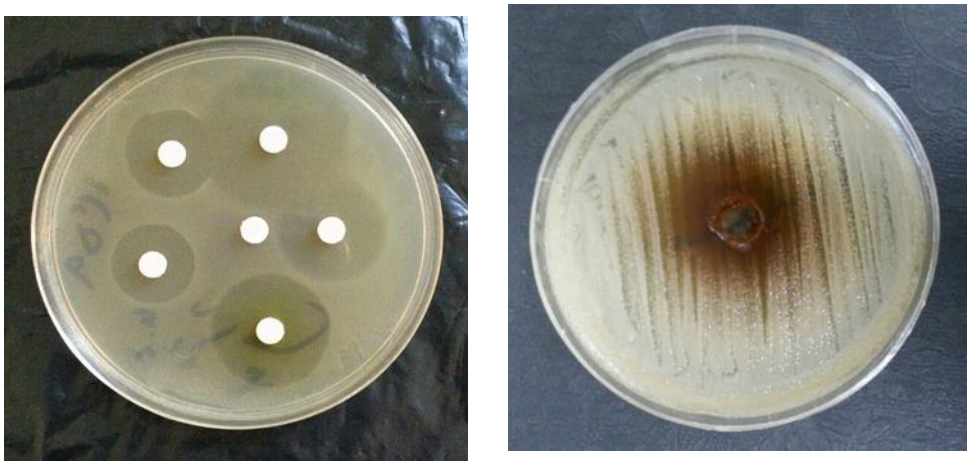

Figure 3: (A) Antifungal activity of Fluconazole (B) Antifungal activity of S. aromaticum against C. Albicans

Discussion

Recently, anecdotal evidence and the traditional use of herbs as medicines provide the basis for indicating which plant extracts may be useful for specific medical conditions. There is a growing trend in medicinal plants and herbal-medicine around this subject to find suitable alternatives to synthetic antifungal drugs, concerning the shortage, cost and other disadvantages of them. In this study, we assessed in vitro antimicrobial activity of S. aromaticum (clove powder) in comparison with Fluconazole, a common synthetic drug used against C.albicans.S. aromaticum, contains a significant percentage of eugenol, which has been particular as a compound that display antimicrobial properties(Ranasinghe, Jayawardena, dan Abeywickrama 2002). According to this study, S.aromaticum exhibits antifungal activity against C. albicans. Statistical analyses proved best results by S.aromaticum (inhibition zone diameter: 17.67 ± 3.55 mm) than Fluconazole (inhibition zone diameter: 15.67 ± 0.577 mm) in well diffusion method and antibacterial activities against E. coli inhibition zone diameter: 15.67 ± 0.577 mm) in disk diffusion method. Concerning the similarity between inhibition zone of both medications, it can be interpreted that this statistically significant difference is caused by a large number of samples (n = 32) of E. coli and (n=28) C. albicans. However, this does not change the fact that this herb. The MIC and MBC of S. aromaticum against E. coli (50mg) and (100 mg) respectively. While the MIC and MFC against C. albicans (25mg) and (50 mg) respectively. Through these results, we observe that C. albicans are less resistant to the cloves than the E. coli.

Conclusion

The S. aromaticum were proved to have antimicrobial activity against E. coli and C. albicans Using the standard agar dilution method, the MIC and MBC of the S. aromaticum against E. coli isolate showed a considerable antimicrobial activity (50 mg), (100 mg) respectively. While the MIC and MFC of the S. aromaticum against C. albicans (25 mg), (50 mg) respectively, shown in (Table 1). Whereas, the diameters of bacterial inhibition zones (mm) measured for E. coli 15.67 ± 0.577 (mm), and the diameters of fungal inhibition zones (mm) measured for C. albicans 17.67 ± 3.55 (mm) compared with Fluconazole as shown in (Table2).The in vitro results of the present investigation give evidence that the clove has a powerful activity of multi antimicrobial properties, especially to pathogenic strains. Since clove displayed a decisive antimicrobial activity, it can be considered a viable alternative to conventional antifungal, antibacterial with a relatively minimal side effect, lesser toxicity and lower cost. The activity of the extract could provide a different manner to overcome a problem of microbial infections caused E. coli and C. albicans can be translated into useful clinical applications in order to raise the pharmacological activity or decrease the resistance behavior of resistant E. coli and C. albicans. There are many proofs indicating that medicinal plants will constitute a prevalent alternative for effective treatment of microbial diseases as an alternative to the costly antibiotics.In conclusion, Moreover, this study showed that the clove bud powder has higher activity in inhibiting the growth of C. albicans than E. coli In fact; the herbs could be alternative substances for fungi control, and especially strains that have acquired resistance to conventional antimicrobial agents. However, further studies are needed to purify the major components of these plants and to assess their appreciable antimicrobial actions against microbial species in vivo. However, if these herbal extracts are to be used for medicinal purposes, issues of safety and toxicity will need to be addressed.

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